SOLE/JOINT

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name: that I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought in the application entitled:

INTEGRATIVE PROTEIN-DNA COCHLEATE FORMULATIONS AND METHOD FOR TRANSFORMING CELLS

which application is:

☐ The attached application (for original application)

☑ filed application Serial No. 09/210,578 December 14, 1998 , and amended on

(for declaration not accompanying application)

that I have reviewed and understand the contents of the specification of the above-identified application, including the claims, as amended by any amendment referred to above; that I acknowledge my duty to disclose information of which I am aware which is material to the patentability of this application under 37 C.F.R. 1.56, that I hereby claim priority benefits under Title 35, United States Code §119, §172 or §365 of any provisional application or foreign application(s) for patent or inventor's certificate listed below and have also identified on said list any foreign application for patent or inventor's certificate on this invention having a filing date before that of any foreign application on which priority is claimed:

Application Number	Country	Filing Date	Priority Claimed
			NO

I hereby claim the benefit of Title 35, United States Code §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in a listed prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge my duty to disclose any information material to the patentability of this application under 37 C.F.R. 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status

I hereby appoint John H. Mion, Reg. No. 18,879; Thomas J. Macpeak, Reg. No. 19,292; Robert J. Seas, Jr., Reg. No. 21,092; Darryl Mexic, Reg. No. 23,063; Robert V. Sloan, Reg. No. 22,775; Peter D. Olexy, Reg. No. 24,513; J. Frank Osha, Reg. No. 24,625; Waddell A. Biggart, Reg. No. 24,861; Louis Gubinsky, Reg. No. 24,835; Neil B. Siegel, Reg. No. 25,200; David J. Cushing, Reg. No. 28,703; John R. Inge, Reg. No. 26,916; Joseph J. Ruch, Jr., Reg. No. 26,577; Sheldon I. Landsman, Reg. No. 25,430; Richard C. Turner, Reg. No. 29,710; Howard L. Bernstein, Reg. No. 25,665; Alan J. Kasper, Reg. No. 25,426; Kenneth J. Burchfiel, Reg. No. 31,333; Gordon Kit, Reg. No. 30,764; Susan J. Mack, Reg. No. 30,951; Frank L. Bernstein, Reg. No. 31,484; Mark Boland, Reg. No. 32,197; William H. Mandir, Reg. No. 32,156; Scott M. Daniels, Reg. No. 32,562; Brian W. Hannon, Reg. No. 32,778; Abraham J. Rosner, Reg. No. 33,276; Bruce E. Kramer, Reg. No. 33,725; Paul F. Neils, Reg. No. 33,102; Brett S. Sylvester, Reg. No. 32,765; and Robert M. Masters, Reg. No. 35,603, my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and request that all correspondence about the application be addressed to **SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC**, 2100 Pennsylvania Avenue, N.W., Washington, D.C. 20037-3213.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date 19 Feb. 1999 First Inventor David MARGOLIS
(First Name / Middle Initial / Last Name)

Residence Baltimore (City) Maryland (State/Country) Signature

Post Office Address: 4506 Roland Avenue, Baltimore, Maryland 21210

Citizenship United States

Date 2/12/99 Second Inventor Susan GOULD-FOGERITE

First Name Middle Initial Last Name

Residence Annandale, New Jersey Signature Susan Gould-Fogerite

City State/Country

Post Office Address: 6 Cynthia Court; Annandale, New Jersey 08801

Citizenship United States

Date 16 Feb, 1999 Third Inventor Raphael James MANNINO

First Name Middle Initial Last Name

Residence Annandale New Jersey Signature Raphael J. Mannino

City State/Country

Post Office Address: 22 Victoria Drive; Annandale, New Jersey 08801

Citizenship United States

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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

David MARGOLIS, et al.

Appln. No.: 09/210,578

Group Art Unit: 1646

Filed: December 14, 1998

Examiner: E. Sorbello

For: INTEGRATIVE PROTEIN-DNA COCHLEATE FORMULATIONS AND METHODS FOR TRANSFORMING CELLS

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Susan Gould-Fogerite, hereby declare and state:

THAT I am a citizen of the United States;

THAT I have received the degree of Doctorate of Philosophy in 1985 from Albany Medical College, Albany, New York;

THAT I have been employed by UMDNJ, New Jersey Medical School, since 1991, where I hold a position as Assistant Professor, with responsibility for teaching in and Co-Directing the Medical School Immunology Course; teaching in the Graduate School Cell Pathology Course and Immunology Course, training Graduate students, and directing Biomedical Research in the areas of Vaccinology and Gene Therapy.

I submit the following statements and scientific evidence to overcome the Examiner's rejections as follows.

As a general background to my statements that follow, I will distinguish the differences between cochleates, as claimed in the present invention, and liposomes.

Classic liposomes are comprised of fluid lipid bilayer membranes with an aqueous space contained within. Such fluid membranes are highly thermodynamically stable. As a result, liposomes tend to resist fusion with other fluid membrane bilayers, therefore, when a liposome, containing a biologically active agent encounters a target cell membrane, little or no direct transfer of the agent occurs. Other general disadvantages of liposomes are outlined on pages 2-3 of the prospectus and on page 2, line 20 to page 3, line 22 of our specification. As a result, other liposomes were generated. I direct the Examiner's attention to the attached article authored by C. Ropert entitled, *Liposomes As A Gene Delivery System*, 32(2) Braz. J. Med. Biol. Res, 163 (Feb. 1999). Here (*see* page 4), cationic lipids typically used for transfection and other gene transfers are described. The most common embodiment of such liposomes is the Lipofectin™ product. However, these liposomes still suffer from an inefficient ability to deliver DNA when administered systemically. The properties and structures of cationic lipids and their complexes with DNA are significantly different from anionic lipid cochleate –DNA complexes.

For an explanation of cochleates as presently claimed, I submit the attached company prospectus: *Historical Perspective: The Origin of Cochleates* that my colleagues and I compiled. Page 1 of the prospectus describes how cochleates are formed. Addition of divalent cations, such as calcium, to negatively charged (anionic) lipids, such as phosphatidylserine, induce the collapse of the lipid bilayers to result in the formation of jelly-roll-like structures or cochleates. Unlike liposomes, cochleates do not have an aqueous interior. This unique structure provides protection from degradation for associated or "encochleated" molecules, such as DNA. All of this information is also disclosed on page 2, line 3 to page 3, line 22 in our specification.

Further, please refer to the attached schematic diagrams depicting how cochleates "encochleate" DNA molecules (plasmids or single-stranded anti-sense) and how cochleates deliver DNA molecules into a cell.

Turning to the 35 U.S.C. § 102(e) rejection over Zychlinsky, the Examiner asserts that Zychlinsky teaches a lipid carrier with DNA. However, I disagree with the Examiner's assertion.

I submit that the heading of Col. 29, line 20 of Zychlinsky is "Cochleates," the text and references cited within this section concern proteoliposomes, not cochleates. On page 2, line 20 to page 3, line 13 of the present specification distinguishes cochleates from liposomes. Therefore, I am of the opinion that the references and text disclosed in Zychlinsky are presented in a manner that is scientifically incorrect and indicates a lack of understanding on the part of the inventors of Zychlinsky.

For example, Col. 29, line 23 of Zychlinsky discloses that "cochleates are used for in vivo DNA transfer." However, the references cited: Gould-Fogerite, S. et al., 1985, Anal. Biochem. 148:15-25; Mannino, R.J. et al., 1988, Biotechniques 6:682-690; and Papahadjopoulos, D. et al., Biochim. Biophys. Acta, 1975, 394:483-491, do not disclose this concept.

Gould-Fogerite, S. et al., 1985, Anal. Biochem. 148:15-25, of which I am an author, describes the "direct calcium" (DC) method for making large, unilamellar liposomes with integrated viral glycoproteins, and the physical and biochemical properties of these vesicles, and not cochleates as presently claimed.

[redacted]

Mannino, R.J. et al., 1988, Biotechniques 6:682-690, of which I am an author, describes *in vivo* gene transfer using fusogenic proteoliposomes containing either Sendai or influenza virus glycoproteins in the lipid bilayer with DNA encapsulated in the aqueous interior.

Papahadjopoulos, D. et al., Biochim. Biophys. Acta, 1975, 394:483-491 discloses a phospholipid cochleate, but does not describe using the cochleate to encapsulate a DNA molecule nor does Papahadjopoulos teach its use as a gene transfer vehicle.

Further, Col. 29, line 36-50 of Papahadjopoulos cites Gould-Fogerite, S. et al., Gene, 1989, 84:429-438, of which I am an author, but does not disclose cochleates. Rather, this article relates to gene transfer using proteoliposomes.

Concerning the Examiner's 35 U.S.C. § 112, first paragraph rejection, I submit that my invention is enabling for *ex vivo* use. I submit that *in vitro* manipulation of hematopoietic stem cells, particularly CD34+ cells, is well known to those of ordinary skill in the art. Further, the capability and success of using such cells *ex vivo* is well known and documented. Therefore, in my opinion, the transduced CD34+ cells disclosed in the present specification are expected to repopulate an animal or human patient to achieve an *ex vivo* effect. In support of this position, I have included several scientific references that report the ability of manipulating hematopoietic stem cells, taken from an animal or human patient, autologously transplanting these manipulated cells and observing an effect therefrom.

Cavazana-Calvo et al., *Gene Therapy Of Human Severe Combined Immunodeficiency (SCID)-X1 Disease*, Science vol. 288 no. 5466, pages 669-672 (April 28, 2000). This reference describes an *ex vivo* autologously transplanted use for CD34+ cells, taken from infant bone marrow, which were transduced with a retrovirus-derived vector containing the gene encoding the gamma cytokine receptor subunit of various interleukin receptors. This study shows that

[redacted]

gamma transgenes and appropriate antigenic-specific responses were detected in two patients, thus demonstrating a clinical benefit for this type of gene therapy. See Cavazana-Calvo et al., attached hereto.

Schiedlmeier et al., *Quantitative Assessment Of Retroviral Transfer Of The Human Multidrug Resistance 1 Gene To Human Mobilized Peripheral Blood Progenitor Cells Engrafted In Nonobese Diabetic/Severe Combined Immunodeficient Mice*, Blood, vol. 95, no. 4 pages 1237-1248 (February 15, 2000). This reference demonstrates successful *ex vivo* gene therapy using human CD34+ cells, transduced with retroviral-mediated cytostatic drug-resistance genes, to repopulate mice. See Schiedlmeier et al., attached hereto.

Parkman et al., *Gene Therapy For Adenosine Deaminase Deficiency*, Annual Rev. Med., vol. 51, pages 33-57 (2000). This reference teaches clinical gene therapy trials for adenosine deaminase (ADA) deficiency using hematopoietic stem cells taken from either umbilical cord blood or neonatal bone marrow and using mature T lymphocytes. These cells were transduced using a murine retroviral-based vector containing the ADA gene and then engrafted into non-myeloablated patients. The authors conclude that hematopoietic stem cells appeared to yield a higher transduction/engraftment ratio than the yield obtained from using mature T lymphocytes. See Parkman et al., attached hereto.

Isner et al., *Angiogenesis And Vasculogenesis As Therapeutic Strategies For Postnatal Neovascularization*, J. Clin. Invest, Vol. 103, No. 9, page 1231-1236 (May 1999). This reference demonstrates that CD34+ cells can appropriately populate and differentiate into endothelial cells when transplanted *ex vivo*. Specifically, Isner teaches the isolation of CD34+ cells from the leukocyte fraction of peripheral blood to obtain putative angioblasts. These cells were taken from a transgenic mouse that constitutively expressed the marker gene, LacZ which

is regulated by one of two promoters, Flk-1 or Tie-2. These were then transplanted into another mouse with a specific ischemia and assayed for β-galatosidase. Isner found that the transplanted CD34+ cells appropriately repopulated and differentiated into endothelial cells. See Isner et al., attached hereto

I am also of the opinion that the Debs et al. reference, cited against Claim 25 under 35 U.S.C. § 103, does not stand for the proposition for which the Examiner asserts. Specifically I am of the opinion that the specific assertion by Debs that "[i]t may be possible to further increase the incidence of transgene integration into genomic DNA by incorporating a purified retroviral enzyme, such as the HIV-1 integrase enzyme in the lipid-carrier DNA complex" (see Col. 9, lines 10-14) is purely theoretical in nature. Further, since the construction of vectors intended for stable genomic integration is not routine, my opinion is that the simple addition of an integrase with a cationic lipid and a plasmid containing long terminal repeats (LTR) will not necessarily result in increasing the incidence of transgene integration into genomic DNA as asserted in Debs. In support of my position, I have conducted an extensive scientific literature search to find any journal articles to support Debs' assertion, and have found none.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 7/26/00

Susan Gould-Fogerite
Dr. Susan Gould-Fogerite, Ph.D.